Sites were initially assigned a random order to be sampled using a random number generator and surveyed from 21 May – 12 July 2001; however, we deviated from this order slightly as rain events created poor sampling conditions at a few sites on the day that site was to be sampled. Methods used to survey mussels were identical to those used to survey 80 sites mentioned in a prior final report to the NCDOT (Project Hwy# 2003-10)(Levine et al. 2003). At each site, three surveyors each searched 1-meter-wide linear transects (one next to each bank and one in the center of the stream) using view scopes and snorkeling to visually locate mussels. These transects were searched in an upstream direction for the entire 600 meters of stream surveyed at each site and under the crossing structure. The 1-meter width was standardized on each surveyor by measuring against their armspan giving each person a reference point on their body by which to measure, and no mussels were included in the survey that fell outside this 1-meter width. As surveyors moved upstream, the 1-meter transects on each bank were measured from the water's edge using the reference point on their armspan, and the transect in the center of the stream was measured from the centerline of the surveyor's body. The same surveyor surveyed the same linear transect (left bank, middle, or right bank) for an entire site, and a standard rotation was used between sites. In larger, more diverse streams, we used 1-2 extra surveyors to qualitatively search areas between the three linear transects to try to find species not accounted for in the linear transects. The qualitative searches also yielded extra data on sex ratios and gravidity of sexually dimorphic species (Lampsilines). All sites but two were completed on the same day. The two sites that required two days were completed in consecutive days, and no substantial weather changes or rain occurred between those days.

To maximize consistency through time and between surveyors, only visual surveys were done, and no excavation or rock flipping was used to locate mussels. Tactile searching was used occasionally as necessary when murky water, debris piles, or undercut banks made visual searches difficult; however, only mussels felt on the sediment surface were taken. Mussels were identified, and length was measured to the nearest millimeter using calipers on the first 15 of each species collected from each cross-section. We recorded the cross-section number (See Chapter 1) and linear transect (left bank, middle, right bank) in which the mussel was located. Lampsilines were classified as male or female, and we checked for gravidity (presence of mussel larvae) of all known females. Mussels were returned to original life position as soon as data was recorded for each individual.

Two specific measures were taken in the field for quality assurance. Between sites we alternated between starting the survey at two different points within the reach to be sampled. At half of the sites, we started the survey at the most downstream end of the site and moved in an upstream direction to sample the entire reach. At the other sites, we started at the road crossing surveying the upstream reach first then going the downstream end and searching up to the road crossing. This was done to guard against a time bias with respect to the road crossing, so the same portion of stream was not always sampled at the same time of day. Also, a measure of detectability was taken at each site in a predetermined 75-meter reach by removing all mussels found in the bank transects and using a second pass by the field supervisor to locate any mussels missed. This provided a measure of variation in mussel detection between days and between surveyors. Detectability percentage was calculated as the number of mussels found in the first pass divided by the total number found in the first and second passes.

All data were tested for normality using a Ryan-Joiner test (Dekker 1986). We then analyzed the data in a variety of ways to assess potential differences in relative mussel abundance and diversity in relation to the road crossing. To equally weight all sites, relative